

REMARKS

Applicant notes with appreciation the detail and thoroughness of the Office Action mailed March 23, 2004. This amendment is submitted in response thereto. In response to the objection to the specification as lacking appropriate headings, the attached substitute specification is submitted to address these objections as well as correct minor typographical errors therein. Claims 1-7 currently stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite with respect to ambiguity as to whether extraction occurs in the first step of the inventive process. Additionally, claim 1 is considered unclear with respect to the term "the region." Additionally, antecedent basis for various terms found in claims 2, 3 and 5 have also been noted (Paper No. 20040320, pages 4-5). In response to this rejection, the pending claims have been amended consistent with the Examiner's proposed changes. In view of these amendments, it is now believed that the rejections with respect to claims 1-7 under 35 U.S.C. §112, second paragraph, have been overcome and withdrawal of the rejection is respectfully requested.

Claims 1-5 and 7 currently stand rejected under 35 U.S.C. §102(b) or in the alternative under 35 U.S.C. §103(a) over Schankereli (US 5,782,914). Lastly, claim 6 stands rejected under 35 U.S.C. §103(a) over Schankereli in view of Druecke et al. (US 6,187,137).

Remarks Directed to Rejection of Claims 1-5 and 7 Under 35 U.S.C. §102(b) or in the Alternative Under 35 U.S.C. §103(a) with Respect to Schankereli

The Examiner is respectfully requested to reconsider the rejection under 35 U.S.C. §102(b) as anticipated by Schankereli as applied to presently amended claims 1-5 and 7. Anticipation has always been held to require absolute identity between the claimed invention and the teachings found in a single reference.

Claim 1 in amended form recites the steps of “dehydrating to a water content in the range of 10 weight percent to 25 weight percent by subjecting the tissue to an extraction with an organic solvent” and subsequently dehydrating by freeze drying.

In contrast to the invention found in pending claim 1, Schankereli only teaches soaking tissue in 70% ethanol solution for several days (column 2, lines 19-26). Applicant submits that no tissue having a water content per claim 1 is afforded by soaking in 70% ethanol. As the ethanol solution is 70% ethanol and the intention of the claimed invention is to dehydrate tissue, it simply is not possible to immerse a wet tissue in 70% ethanol and after any amount of time obtain a tissue having a water content from 10 to 25 weight percent.

Additionally, the invention of claim 1 differs from Schankereli in subjecting the partly dehydrated tissue obtained from solvent extraction to freeze drying. Applicant respectfully submits that freeze drying is not an equivalent process step relative to vacuum drying according to Schankereli. Freeze drying is a process wherein a substrate to be dried is first deep frozen such that water contained within the substrate is frozen and thereafter a high vacuum is applied to sublimate ice directly to water vapor. In contrast, vacuum drying according to Schankereli occurs at a temperature between 22 and 27° C and as such the water contained within the substrate is liquid resulting in a liquid to gas phase transformation (column 3, lines 37-41).

On the basis of the above amendments and remarks, it is respectfully requested that the rejection of claims 1-5 and 7 under 35 U.S.C. §102(b) be withdrawn.

**Remarks Directed to Rejection of Claims 1-5 and 7
Under 35 U.S.C. §103(a) Over Schankereli**

Claims 1-5 and 7 are rejected as being obvious over Schankereli in the alternative on the basis that the difference between the claimed invention and the teachings of Schankereli are so

slight as to render the claims obvious. It is the Examiner's position that one of skill in the art would be motivated by Schankereli to carry out the claimed invention in order to prepare tissue.

Applicant submits that Schankereli fails to teach the source of the problem that the present invention solves. Schankereli provides a dried, sterilized tissue which is rehydrated at the time of use in sterile saline so as to exhibit the same physical properties as prior art products thereto that had previously been packaged in a vile containing a 1% propylene oxide solution as a sterilant, but without the drawbacks associated with tissue storage in a wet state (column 1, line 40 – column 2, line 4).

To provide a dry equivalent to wet storage in propylene oxide solution, Schankereli teaches a process wherein after the cleaning operation of the tissue the following steps are conducted: chemical cross-linking the tissue with a glutaraldehyde solution, soaking the tissue in a 70% ethanol solution for a longer period (10 hours to several days) to remove bioburden and to kill any microbes, rinsing away the process chemicals used in the preceding step, vacuum drying the tissue, packaging and sterilizing the tissue (column 2, lines 15-35). The vacuum drying is conducted at a temperature in the range from 22 to 27° C (column 3, lines 32-41). During this drying operation the moisture content of the tissue is decreased from about 70% to about 10% by weight (column 3, lines 45-49). Optionally the tissue can be dehydrated in an ethanol solution after the cleaning step and before the chemical cross-linking step (column 3, lines 11-15).

Since the tissue subjected to the vacuum drying in the Schankereli process has an essentially higher water content than the tissue subjected to the freeze drying in the present process and since the vacuum drying does not dehydrate the tissue as gentle as the freeze drying, the Schankereli process does not solve the problem underlying the present invention, i.e. the

conservation of the native structure of the tissue (see page 3, lines 15-18 of the present description).

The following steps of the Schankereli process show that the conservation of the native structure is not intended. The tissues can be frozen at first (column 2, line 65 – column 3, line 1). This will result in the formation of ice crystals in the tissue and therefore in loosening of the fiber structure. Moreover, the tissue is cross-linked by the treatment with glutaraldehyde. After this treatment the tissue does not have the native structure but a leathery character with reduced suppleness and prolonged resorption time.

Since Schankereli does not identify the problem of how to maintain the native structure of tissues, this reference does not give any hint how to solve this problem.

In view of the above remarks, it is respectfully requested that the rejection of claims 1-5 and 7 under 35 U.S.C. §103(a) over Schankereli be withdrawn.

**Remarks Directed to Rejection of Claim 6 Under
35 U.S.C. §103(a) Over Schankereli in View of Druecke et al.**

Schankereli is cited for teaching all the limitations of base claim 1. It differs from claim 6 in failing to disclose mechanical means as part of the treatment step. Druecke is cited to bolster the teaching of Schankereli with respect to the use of dehydrating through the use of a sonic roller, shake roller or vibrator roll (column 3, lines 38-51). (Paper No. 20040320, page 6).

Applicant submits that claim 6 in current form is allowable on the basis of dependency from claim 1 which is now believed to be in allowable form. Additionally, Applicant submits that the dehydration of an aqueous suspension associated with papermaking fibers is inapplicable to dehydration of biological tissues having intricate passages that most certainly would be

damaged by integrating the vibration, shaking or ultrasonic actions taught by Druecke et al. with the procedure of Schankereli.

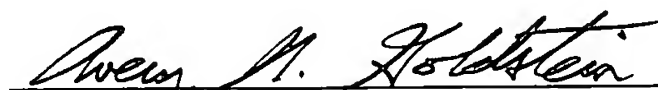
As a result, Applicant submits that the combination of Schankereli and Druecke et al. fails to afford a resultant biological tissue that is comparable to that obtained through the practice of the claimed methodology.

In view of these remarks, it is respectfully requested that the rejection of claim 6 under 35 U.S.C. §103(a) over Schankereli in view of Druecke et al. be withdrawn.

Summary

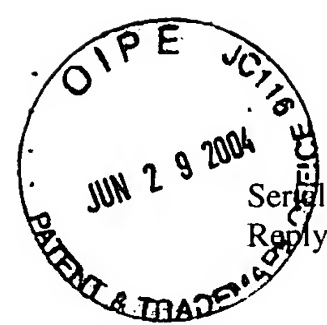
Claims 1-7 are pending in this application. In light of the above amendments and remarks, it is submitted that all the pending claims are directed to allowable and patentable subject matter. Allowance of these claims and the passing of this application to issuance are solicited. Should the Examiner find to the contrary, it is respectfully requested that the undersigned attorney in charge of this application be contacted.

Respectfully submitted,



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A METHOD FOR DEHYDRATING BIOLOGICAL TISSUE FOR PRODUCING PRESERVED TRANSPLANTS

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application is the U.S. National Phase of PCT/EP00/07078 filed July 24,
2000, which in turn claims priority of German Patent Application DE 199 40 426.7
filed August 26, 1999, the contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to a method for dehydrating biological tissue for
producing preserved transplants.

10 BACKGROUND OF THE INVENTION

Methods for the dehydration of biological tissue for producing preserved
transplants deliver autografts, allografts or xenografts which are available to the
surgeon at any time as required.

15 Transplants should have a morphological structure very similar to the native
tissue, for example skin, tendons, bones and their properties should largely
correspond to those of the native tissue. The required properties include internal
surface, handling capability and elasticity. Furthermore, still further criteria must also
be observed in the producing of preserved transplants. The transplant must be able to
be stored in a sterile condition for practically any length of time while maintaining its
20 properties. It must furthermore have a certain resistance to degradation by the
receiving organism so that it can function as a guide rail for tissue sprouting in.

A known method for dehydrating biological tissue for producing preserved transplants makes use of freeze drying. The aqueous tissue is frozen at approximately -25°C to -40°C and the ice which arises is removed by sublimation in a vacuum. The resulting tissue has a low water content. It can be stored in a sterile condition for a long period while maintaining its properties and is available, when required, as a ready-to-use preserved transplant.

This method is, however, connected to disadvantages. With areal collagenic tissue, for example with dura mater, relatively thick spongiform materials are created which makes their handling more difficult. The collagenic starting tissue is a swollen fibrous network in the moist state and this state is fixed during deep freezing. The ice crystals which form between the fibers and the fibrils during freezing loosen the fiber structure. During the subsequent sublimation, cavities arise in the tissue which degrade its properties in comparison with the native tissue. In particular the elasticity is substantially degraded. Furthermore, as a result of partial bonding of the fibrils, the inner surface is dramatically degraded. The resulting product thereby only has a greatly reduced guide rail effect for inwardly sprouting connective tissue when used as a transplant.

Due to these disadvantages of freeze drying, a method is described in DE 29 06 650 C2 in which the collagenic tissue is dehydrated with an organic solvent which can be mixed with water. In this method, a gradual de-swelling of the biological tissue takes place during the successive extraction of the water so that the native fibrillary structure is maintained and no bonding of the fibrils occurs. Consequently, the inner surface of the tissue dehydrated in this way corresponds to

that which the native tissue has. The elasticity is likewise substantially maintained. In this method, however, a number of extraction steps are required for the far-reaching dehydration in which the solvent has to be replaced over and over again. With spongiosa bones up to 20 extraction steps are required. This represents a time-consuming process. The frequent solvent changes are also labor and cost intensive. Furthermore, an environmentally friendly recycling method is required for the solvent.

A method is described in DE 38 35 237 C1 in which bovine pericard tissue is dehydrated with acetone, dried in air, rehydrated with water and then freeze dried. First, this method is relatively complex and, second, the same disadvantages occur as were described above with the method of freeze drying, since the rehydrated tissue is freeze dried.

SUMMARY OF THE INVENTION

The object of the present invention is the providing of a method for dehydrating biological tissue for producing preserved transplants in which the native structure of the collagenic tissue is largely maintained, on the one hand, and which is less time-consuming and less labor and cost intensive, on the other hand.

To satisfy this object, a method is provided for dehydrating biological tissue for producing preserved transplants in which, in a first step, the tissue is partly dehydrated with an organic solvent which can be mixed with water and, in a second step, the tissue is further dehydrated by freeze drying.

It is possible with this two-step method to achieve a faster dehydration, which is also more favorable under labor and cost aspects, while simultaneously maintaining

the native structure, in particular the inner surface, and the elasticity of the collagenic material. The number of extraction steps with the organic solvent can be considerably lowered in comparison with a preservation method in which dehydration takes place only with organic solvents. The preservation process is thereby
5 considerably shortened, solvent is saved and, consequently, less solvent is led to recycling and also frequent, labor-intensive changing of the solvent with added water for fresh solvent is saved. The freeze drying step additionally provides the advantage of simple handling due to the drying with fully automated apparatuses. The initially named disadvantages of freeze drying surprisingly do not occur with the method in
10 accordance with the invention.

DETAILED DESCRIPTION OF THE INVENTION

Human or animal tissue can be used as the biological tissue in the method of the invention, for example skin, dura mater, fascia lata, tendons, vessels, cartilage, pericard, bones and plates made of bone, nails, pins, screws. This tissue consists of
15 collagen or of collagen and mineral components. The transplants produced in accordance with the method of the invention are available to the surgeon at any time.

The tissue is preferably dehydrated in the first step with the organic solvent which can be mixed with water to a water content in the range from 10 weight percent to 25 weight percent. With soft tissue such as skin, dura mater, fascia lata,
20 dehydration is preferably carried out to a water content in the range from 17 weight percent to 20 weight percent. With hard tissue such as bone, in particular spongiosa bone, dehydration is preferably carried out to a water content in the range from 10 weight percent to 15 weight percent, with, as is to be expected, the structure of the

native tissue being maintained. However, it is surprising that the subsequent freeze drying in the second step for the further dehydration up to the desired water content of less than 8 weight percent, which is as low as possible, does not have a negative effect on the tissue structure.

5 Methanol, ethanol, propanol, isopropanol, acetone, methyl ethyl ketone or their mixtures can be used, for example, as solvents in a manner known per se. Preferably, acetone is used ~~[[a]]~~ as an organic solvent which can be mixed with water. The solvent used should have a water content which is as low as possible, it should preferably be free of water. The dehydration with the solvent is carried out at
10 temperatures in the range from 0°C to 70°C depending on the solvent used. The dehydration of the first step preferably takes place at room temperature.

 The tissue is preferably exposed to a vacuum after the dehydration of the first step before it is deep frozen to temperatures from approximately -25°C to -40°C. The organic solvent is thereby largely removed from the tissue.

15 In the dehydration in particular of spongiosa bone, it can be advantageous to simultaneously carry out a treatment with ultrasound, vibrators or rockers in the first step during the dehydration with the organic solvent. This promotes a better penetration of the solvent into the fine passages of the spongiosa bone and thereby the degreasing and the dehydration. For the same purpose, an overpressure, alternating
20 pressure or underpressure can also be applied. It can furthermore be advantageous to carry out a vacuum treatment before the first extraction step and after every extraction step before dehydration is carried out with fresh solvent in the next step. This also promotes degreasing and a better exchange of the aqueous organic solvent in the

passages with fresh organic solvent. All these measures can also be carried out with soft tissues.

The freeze drying in the second step takes place in a conventional freeze drying unit. The partly dehydrated tissue is therein gradually brought to temperatures from, for example, -25°C to -40°C and the ice produced in the tissue is removed by sublimation by applying a vacuum. As already stated further above, a vacuum is preferably applied before the freeze drying, that is before the cooling of the tissue to low temperatures. In this way, the solvent is removed from the tissue in part. The freeze drying follows on from this.

The invention will be explained in more detail with reference to the following examples and to Figures 1 and 2. Figures 1 and 2 are diagrams in which the time curve of the dehydration of the tissue in the examples is shown. The time in hours or days is entered on the abscissa and the water content in weight percent relative to the total weight of the material to be dehydrated on the ordinate.

Example 1

Dura mater is removed from the human body and liberated in a manner known to one skilled in the art from antigenic substances and enzymes. For preservation, the tissue parts cleaned in this manner are treated twice for six hours at a time by being placed into anhydrous acetone at room temperature. The solvent quantity amounts in each case to 500% of the wet weight of the tissue, with a de-swelling of the tissue taking place from 0.65 mm to 0.57 mm. The water content at the completion of this first dehydration step amounts to 20 weight percent.

In the second step, the tissue is cooled for three hours to -40°C in a freeze drying unit. Then, a vacuum of 1.2 mbar is applied for removing the ice formed in the tissue by sublimation. The shelf temperature amounts to 35°C . The water content after the second step, which takes a total of 15 hours, amounts to 6 weight percent. The thickness of the tissue amounts to 0.54 mm and the inner surface is $20\text{ m}^2/\text{g}$. The course of dehydration is shown in Figure 1.

After packing in moisture-tight pouches and after sterilizing with gamma rays with a minimum dosage of 15 Kgry, the preserved dura mater can practically be stored without limitation and is ready for use for transplants.

If, ~~instead~~ instead, the dehydration of the dura mater is carried out only with acetone to the same water content of 6 weight percent, three extraction steps of 12 hours each must be conducted.

Example 2

A spongiosa bone is prepared in a suitable manner known to one skilled in the art. For preservation, the prepared bone is treated five times for 24 hours each time with anhydrous acetone at room temperature. The solvent quantity amounts to 500% of the wet weight of the bone in each case. After this treatment, the water content amounts to 12 weight percent. The bone is subsequently cooled for 3 hours to -40°C in the second step and then a vacuum of 1.2 mbar applied for removing the ice formed in the bone by sublimation. The shelf temperature amounts to 35°C . The water content after the second step, which takes a total of 48 hours, amounts to 2 weight percent. The dehydration course is shown in Fig. 2.

If, instead, the dehydration of the spongiosa bone is carried out only with acetone, then up to 20 extraction steps of 24 hours each are required, with approximately 60 ltrs. of acetone being ~~require~~ required per 1 kg of bone.